Oxygenation in Anemic Newborn Lambs with High or Low Oxygen Affinity Red Cells

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Summary

Experiments to determine the effect of the oxygen-hemoglobin dissociation curve on oxygen delivery during neonatal anemia, were carried out on 15 sedated and ventilated lambs less than 48 hr of age. Eight of the animals were exchange transfused with adult blood. The P_{50} of the exchange transfused group was 32.1 mm Hg (low O_2 affinity) compared to 19.4 mm Hg for the controls (high O_2 affinity). The animals were made anemic by isovolumic exchange transfusions with plasma. At different levels of hemoglobin defined as mild (8 mg/100 ml), moderate (6 mg/100 ml), and severe (4 mg/100 ml) anemia, tissue oxygenation, hemodynamic status, and blood gases were compared. Mixed venous PO_2 was significantly lower in the high affinity group throughout the study. Cardiac output was significantly greater in the low affinity group during severe anemia. Oxygen consumption remained stable in the low affinity group, but decreased significantly in the high affinity group when the anemia was severe. The data indicate that during severe anemia, blood with a high P_{50} is more capable of adequately oxygenating tissues than that with a low P_{50} .

Speculation

Early preterm newborn infants may not adequately oxygenate their tissues during anemia because of their high oxygen affinity red cells.

An important adaptive mechanism during adult anemia is the shift to the right of the oxygen hemoglobin dissociation curve (15). Early preterm newborn infants have red cells with a relatively high oxygen affinity (3) and besides the numerous neonatal complications of prematurity these infants can also have a fall in hemoglobin level. It is apparent that a study was required to determine whether there could be obvious physiologic disadvantages of being anemic during a period of life when red cells have a high affinity for oxygen.

In order to answer this question, studies were planned using the newborn lamb, within the first 48 hr of life, as an experimental model. The newborn lamb is born with a P_{50} which is similar to that of an early preterm newborn infant (18-19 mm Hg) (1) while the P_{50} of the adult sheep blood is considerably higher (30-38 mm Hg) (10). This study was designed to compare at different degrees of anemia, tissue oxygenation, hemodynamic status as well as blood gases in newborn lambs with either high or low oxygen affinity red cells.

MATERIALS AND METHODS

Fifteen newborn lambs less than 48 hr old were included in the study. Eight lambs containing high oxygen affinity red cells were considered as the high O_2 affinity group and seven lambs who underwent a two volume exchange transfusion (160 ml/kg) using freshly drawn maternal blood made up the low oxygen affinity

group. The animals were prepared in conformance with the guiding principles in the care and use of animals as recommended by the Declaration of Helsinki. They were first sedated enough to be kept asleep with repeated subcutaneous injections of Diazepam (0.3 mg/kg every 20-30 min). To eliminate sensibility to pain during catheter position, lidocaine hydrochloride (2%) was used as a local anesthetic. Polyethylene catheters were then positioned, in the right atrium and the ascending aorta, using both a femoral vein and a femoral artery. Similarly using the remaining femoral vein a flow-dependent balloon catheter (Swan-Ganz 5F) was placed in the pulmonary artery. All animals were mechanically ventilated, after endotracheal intubation, by means of a Harvard animal respirator (Millis, MA) with the tidal volume and frequency adjusted so that the end-tidal PCO_2 was 35 ± 5 mm Hg. Temperature was kept constant at $38^\circ C \pm 0.5$. Only as a means of insuring comparable constant metabolic conditions, they all received an intravenous injection of tubocurarine chloride (0.3 mg/kg). A quarter of this initial dose was then repeated every 30 min.

In order to produce a gradual isovolumic anemia in the two groups of animals, exchange transfusions were done with fresh plasma (obtained from sheep) reducing the hemoglobin level in g/100 ml, in three progressive stages, firstly from the control level of 12 to 8 (mild anemia), then to 6 (moderate anemia), and finally to 4 (severe anemia). Before beginning the plasma exchange transfusion and 30 min after each stage of anemia, arterial and mixed venous PO_2 , PCO_2 , pH, and oxygen hemoglobin saturations were monitored using a IL Blood Gas Laboratory 217 (Instrumentation Laboratories, Lexington, MA). Oxygen content was measured in duplicate samples from the aorta and pulmonary artery, using a Lex- O_2 -Con (Lexington Instrument Corporation, Waltham, MA). The accuracy of O_2 content values measured directly on the Lex- O_2 -Con was checked with the calculated values based on the oxygen saturation obtained by the Co-Oximeter. In all instances they agreed within 0.2 vol %. Throughout the study the difference between the O_2 content of the pulmonary artery and the right atrium was never greater than 2%, indicating that there was no left to right shunt through the ductus arteriosus.

The oxygen affinity was expressed as P_{50} , the oxygen tension at which hemoglobin is half-saturated. This was determined on fresh blood samples equilibrated in a tonometer for 30 min at $37^\circ C$, which permitted equilibration and sampling of successive aliquots of blood at different O_2 tensions. Measurements of O_2 saturation pH and PO_2 provided the information for determining the P_{50} which was converted to pH 7.4 with a Bohr effect of 0.46 (7). There were at least four experimental points for each oxygen-dissociation curve. The S.D. of the mean P_{50} obtained on the same blood sample was 0.76 mm Hg ($n = 11$).

Aorta and pulmonary artery pressures were continuously recorded on a Grass model 7B Polygraph using a Statham strain gauge (P23Dc) transducer. To measure oxygen consumption, expired air was collected in a spirometer for a period of 3 min. This procedure was repeated 7-8 times per animal and the S.D. of the minute-volume was always less than 2% of the mean. The oxygen concentration in the expired gas was measured directly using an

oxygen analyser (model S-3A, Applied Electro-Chemistry Inc., Sunnyvale, CA).

The oxygen consumption ($\dot{V}O_2$) as well as all blood samples were studied in duplicate after each decrease in hematocrit level and from these data, the cardiac output of each animal was calculated using the Fick principle. Twelve normal newborn lambs were used to validate the measurements of $\dot{V}O_2$ obtained in this study. In these animals $\dot{V}O_2$ was measured four times during an interval of 6 hr and the results showed little variations (S.D. of the values was less than 5% of the mean).

The statistical test used to compare the mean data obtained in the study was the Student's two sample *t* test. However when the difference in $\dot{V}O_2$ within the same high O_2 affinity group was evaluated (control level of hemoglobin *versus* severe anemia) a paired *t* test was used. Hotelling's T^2 multivariate analysis of tests (18) were used to compare the mean $\dot{V}O_2$ obtained at the four levels of hemoglobin of both groups of animals. Also a nonparametric Mann-Whitney test (13) was used to check the normality assumption of the same data.

RESULTS

The results of these studies are expressed as mean and S.E. The control pH values for the high and low affinity groups were 8.394 ± 0.034 and 7.438 ± 0.037 , respectively. These pH values did not change significantly during the study. The mean P_{50} of each of the two groups of lambs at the control levels of hemoglobin were 19.4 ± 1.06 mm Hg for the high oxygen affinity group and 32.1 ± 2.5 mm Hg for the low oxygen affinity group respectively ($P < 0.001$). The P_{50} values of each of the groups were unchanged at the end of the experiment (20.4 ± 0.8 *versus* 32.7 ± 2.8). The relative stability and similarity of the experimental preparation is demonstrated on Figure 1, where the arterial PO_2 of both groups of animals is represented. There was no significant difference in the arterial PO_2 of both groups of animals at the normal hemoglobin level as well as at any stage of anemia. However the mixed venous PO_2 (PvO_2) was significantly lower in the high oxygen affinity group at the control basal level ($P < 0.01$). As the level of hemoglobin decreased in both groups of animals the PvO_2 de-

creased concomitantly and the difference between both groups remained significant throughout the experiment. In severe anemia, the tissue O_2 extraction increased. The percentages of O_2 extraction augmented from 32 to 65% and from 39 to 63% for the high and low oxygen affinity group, respectively. The arterial blood content and mixed venous oxygen content diminished gradually and significantly ($P < 0.01$) as a result of the decrease in hematocrit (Fig. 2). The change was of the same magnitude in both groups of animals.

Under normal control hemoglobin levels there was no significant difference in stroke volume, heart rate, and cardiac output of both groups of animals (Fig. 3). During mild anemia there was an increase in heart rate. As the anemic became more severe both groups of newborn lambs developed bradycardia. Despite this initial rise in heart rate the stroke volume increased slowly. This change in stroke volume was significantly greater during the period of bradycardia (Fig. 3). When cardiac output was correlated to the degree of anemia it was found to increase more significantly in the low oxygen affinity group, while in the very severe anemic state it was more than twice that of the control condition. On the other hand, the cardiac output of the high oxygen affinity group rose slowly reaching a maximum of 6 g hemoglobin and then leveling off between 6 and 4 g hemoglobin.

The mean aortic blood pressures during progressive anemia can be seen in Figure 4 (*top*). There was no difference between the two groups of animals but during severe anemia there was a small decrease in mean arterial pressure in both groups. The pattern was similar with the pulmonary arterial pressures. When the systemic and pulmonary resistance was calculated, a great variability in individual values from one animal to the other was observed. However the mean values, although appearing greater in the high affinity group at all levels of anemia, was not significantly different (Fig. 4, *bottom*).

Figure 5 demonstrates the stability of $\dot{V}O_2$ in the group of newborn lambs exchange transfused with adult blood, even during extreme anemia. However, in the high affinity group a comparable $\dot{V}O_2$ was present only down to 6 g/100 ml hemoglobin. From then on, as the hemoglobin values decreased, oxygen consumption decreased to reach levels significantly lower than those found with

Fig. 1. The arterial and mixed venous PO_2 during progressive anemia. The lines join the mean $\pm$ S.E. of newborn lambs with either high affinity or low affinity red cells. The notations + and ‡ represent significant differences between control and anemic hemoglobin levels, while * indicates a significant difference between high and low affinity blood at all hemoglobin levels.

Fig. 2. The decline in arterial and venous blood oxygen content with progressive anemia, in newborn lambs with either high affinity or low affinity red cells. The lines join the mean $\pm$ S.E. The notations * and ‡ represent significant differences between the hemoglobin levels in control and severe anemia.

Fig. 3. The relationship of stroke volume, heart rate, and cardiac output to progressive anemia. The lines join the mean $\pm$ S.E. of newborn lambs with either high affinity or low affinity red cells. The notation * indicates significant differences in values obtained at different hemoglobin levels and ‡ between the groups having high or low oxygen affinity blood.

adult red cells ($P < 0.01$). There was also a significant decrease of $\dot{V}O_2$ within the high O_2 affinity group during severe anemia ($P < 0.005$).

In order to test the differences between the $\dot{V}O_2$ of the high O_2 affinity group and the low O_2 affinity group, Hotelling's T^2 multivariate test (18) was used incorporating the four levels of hemoglobin. This test indicated that the overall $\dot{V}O_2$ of the low P_{50} group was lower than the high P_{50} group ($P = 0.0287$). To determine what levels of anemia accounted for the difference, variance analysis (F tests) were carried out (18). The $\dot{V}O_2$ differences at 6 g/100 ml hemoglobin gave a P value of 0.08 (nonsignificant) and a significant P value of 0.017 at the level of 4 g/100 ml hemoglobin. These last F tests were then checked for a possible departure from the normality assumption by the nonparametric Mann-Whitney test (13) and yielded P values of 0.076 and 0.012 at 6 and 4 g/100 ml hemoglobin, respectively. Thus the above described treatment of the $\dot{V}O_2$ data confirmed that there was a significant decrease in $\dot{V}O_2$ in the low P_{50} group during severe anemia.

DISCUSSION

Early preterm newborn infants undergo a decrease in their hemoglobin levels during the early weeks of life. During this period these infants are considered to have a refractory anemia and reach very low levels of hemoglobin before resumption of active erythropoiesis (6, 12). Thus infants born at less than 32 wk

Fig. 4. The mean systemic arterial pressure and systemic resistance during progressive anemia with either high or low affinity red cells. The lines join the mean $\pm$ S.E.

of gestation, at the time of their lowest hemoglobin concentration (3–11 wk after birth) have P_{50} values much lower (20.3 ± 2.3 mm Hg) (17) than what has been reported during adult anemia (30.2 ± 1.9 mm Hg) (15). In contrast, some newborn mammalian species that are used as experimental models, rapidly decrease their red cells oxygen affinity soon after birth (1, 2), so that when their postnatal decrease in hemoglobin concentration occurs their P_{50} levels are similar to the adult values (8). In this study an attempt was made to determine if the position of the oxygen-dissociation curve in newborn lambs with high oxygen affinity red blood cells would impair oxygen delivery to the tissues during increasing levels of anemia.

Previous studies (4, 16) have shown that tissue oxygenation, in presence of blood with high oxygen affinity and normal hematocrit, results in a lowering of $P\bar{V}O_2$. In the present experiment, this was similarly observed at control hemoglobin levels. In severe anemia, since the cardiac output has been found to be significantly lower in the group with high affinity red cells compared to adult blood, an increase in the arteriovenous O_2 content difference would be needed to maintain equivalent O_2 delivery to the tissue. The fact that the arteriovenous O_2 content difference did not increase resulted in the decrease in O_2 consumption in the low P_{50} group during severe anemia.

An important factor responsible for this increase in systemic flow during anemia is most likely the decrease in blood viscosity (5, 9). In this study, peripheral resistance fell with anemia and showed a tendency to be greater (though not significantly) in presence of high O_2 affinity red cells. This phenomenon could be one of the factors responsible for the lower cardiac output found in the high affinity group in severe anemia. Unfortunately the data collected in our study do not indicate whether the disparity in peripheral resistance and cardiac output between the two groups of animals was due to vasomotor or viscosity factors. In any case, according to accepted concepts (11), the hemodilution during the phases of moderate and severe anemia of these studies should have similarly improved the flow properties of the blood thus resulting in minimal rheological differences between the two groups of animals.

In our experiments, newborn animals having low oxygen affinity red cells had their arterial O_2 content reduced by severe anemia to a point where $P\bar{V}O_2$ was considered barely adequate for tissue

Fig. 5. The oxygen consumption of newborn lambs with either high affinity or low affinity red cells, in relation to anemia. The lines join the mean and $\pm$ S.E. The notation * indicates a significant difference between the mean values obtained from the high and low oxygen affinity blood groups. The symbol ++ indicates a significant difference between the mean values obtained at the control hemoglobin level and severe anemia within the high oxygen affinity group.

oxygenation. Other newborn animals under the same conditions of severe anemia but having red cells with high oxygen affinity had an even greater decrease in their oxygen consumption which could indicate that an inadequate amount of oxygen was being delivered to their tissues. The recently completed study by Lister *et al.* (8) where nonsedated newborn lambs were evaluated as to oxygen delivery during the first 2 months of life, suggested that there exists a lower mixed venous PO_2 in lambs which is close to some "critical end-capillary PO_2 " where cardiac output would have to increase out of proportion to meet tissue O_2 demands. Although our study was confined to the newborn period and the experimental conditions were not the same, it is of interest to note that the critical low hemoglobin concentration speculated by Lister *et al.* (8) at 6 g/100 ml, is similar to the hemoglobin value below which our data demonstrated a remarkable increase in cardiac output in the low affinity group as well as the decrease in O_2 consumption in the newborn animals with high affinity red cells. As arterial O_2 content dropped oxygen consumption was maintained in part, in the high P_{50} animals, by the increase in cardiac output.

It has been previously shown in a study of infants who were born prematurely, that at comparable levels of anemia, those having high amounts of fetal hemoglobin and thus increased hemoglobin oxygen affinity, manifested higher plasma erythropoietin activity than the infants with higher concentrations of adult hemoglobin and lower hemoglobin affinity (14). This finding led to the speculation that the ability to unload oxygen to the tissues during anemia of prematurely born infants is a function of the position of the oxygen-dissociation curve. The present experiments confirmed this point by demonstrating that in newborn lambs red cells with a high P_{50} were more capable of adequately oxygenating tissues than red cells with low P_{50} during severe anemia. While one cannot extrapolate directly to the early preterm newborn infant, it does suggest that the human premature infant may be at more of a physiologic disadvantage than an adult when oxygen carrying capacity of blood is decreased by anemia. This finding could be clinically important during the neonatal period.

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